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Effect of fermentation on the microbial growth and proximate composition of water yam

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ABSTRACT:
Water yam *Dioscorea alata* sample were subjected to liquid substrate natural fermentation at $30^0C\pm2^0C$ for 120 hours. The total viable counts of the associated microorganisms, pH, and proximate composition of raw (unfermented) and fermented samples were determined. The bacterial isolates identified were *Bacillus subtilis, Staphilococcus aureus, Pseudomonas aureginosa, Bacillus licheniformis, Aerococcus viridians, Leuconostoc mesenteroides. While the fungi species identified were Penicillium italicum, Fusarium solani, Aspergillus fumigatus, Aspergillus flavus and Fusarium Oxysporium.* The bacteria counts obtained from the raw unfermented sample was $3.5x10^4$cfu/ml, however, as fermentation progressed, bacterial load increases till the termination period and the value ranged between 2.7-1.2 x 10^4cfu/g. However, an increase in fungal count was noted as fermentation progressed and the value ranged between 7.0-10.0 x 10^4 spore/g with the sample fermented for 120 h recording the highest microbial load. Protein content of the fermented sample increased significantly ($p\leq0.05$) from 5.76 (raw sample) to 10.34 (fermented sample). The lipid, Ash, crude fiber and carbohydrate content decreased with fermentation till the termination period. The values ranged between 6.31 and 3.41 for lipid, 4.91 and 3.11 for ash, 9.31 and 3.44 for crude fiber and 70.03 and 80.41 for carbohydrate. However, fermentation of the water yam *Dioscorea alata* has greatly helped in the improvement of nutrient composition especially the protein.

Key words:- Water yam, Fermentation, Viable count, proximate, microbial

INTRODUCTION

Yam (*Dioscorea* spp) are climbing plants with glaborous leaves and twining stems, which coil readily around a stake. They are perennial through root system but are grown as annual crops. Yams are undoubtedly a major staple food for most parts of Africa. These are reported to be the most important crop in West Africa. There are many different edible species of yams grown in Nigeria. The varieties of yams grown in Nigeria may be recognized by the range and colour of their leaves and tubers as well as by the direction of their stem twinning as they climb [1]. However, among different varieties, the white yam (*Dioscorea rotundata*) is the most popular in Nigeria. It is reported to be rich in soluble carbohydrate and contain valuable non starchy nutrients. Its digestibility is also known to be high [1]. The water yam (*Dioscorea alata*) on the other hand is known to be very high yielding with high moisture content. It has many varieties, which are recognized by colour and shape and water yam is higher in protein and mineral content than white yam.

In Nigeria, yams are generally consumed in many different forms as food for man while it can also be used as animal feed. This use is reportedly limited only for economic reasons [2]. Yams are essentially for consumption and are eaten, chewed or swallowed, but boiling and pounding are preferred methods of eating yams [2]. Yam storage losses are usually attributed to post harvest pathogens, which include bacteria, fungi, nematodes and insect pests. Many fungal pathogens have been associated with deterioration of yam during storage. The implications of *Fusarium oxysporium*, *Fusarium solani*, *Penicillium* and *Aspergillus* species as common pathogens in yam storage have earlier been reported [3].

Fermentation in food processing typically refers to the conversion of sugar to alcohol using yeast, bacteria, or a combination thereof, under anaerobic conditions. Fermentation usually implies that the action of the microorganisms is desirable, and the process is used to produce alcoholic beverages such as wine, beer, and cider. Fermentation is also employed in enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates, preservation to create lactic acid in sour foods such as pickled cucumbers, kimchi and yogurt [4]. In view of the above facts the present study was conducted to evaluating the effect of fermentation on the microbial load, type of microorganisms and proximate composition of water yam (*Dioscorea alata*)

MATERIALS AND METHODS

The water yam used for this study was bought from Lafenwa market, Abeokuta, Ogun State.

Preparation of samples

Unfermented sample of the water yam (*Dioscorea alata*) was prepared by weighing 1g of the yam sample that was peeled and thoroughly washed with sterile distilled water. Microbial load, characterization of microorganism present and determination of parameters such as the pH and proximate composition were carried out by method of [5]. Fermented samples were prepared by weighing 500g of the peeled and

cleaned water yam into five portions and each sample was submerged into 500 ml of clean sterile distilled water in a separate sterile plastic container and labeled as day 1 to day 5. The plastic container was properly covered for liquid substrate fermentation to take place at room temperature over a period of 0, 24, 48, 72, 96 and 120 hours respectively. The population of different microorganisms was counted and their isolation, characterization was done by the method of [6] and determination of different parameters such as pH and proximate composition were carried out by method of [5]. Microbial population and their isolation were carried out using serial dilution and pour plate methods. The bacterial population was counted on the nutrient agar medium and fungal population on Saboruad Dextrose Agar (SDA) medium. The resultant bacterial isolates after incubation period of 24 h at 37^0C were characterized and identified according to the method of [6] and fungal isolates after an incubation period of 3-5 days at 27^0C were identified on the basis of cultural and morphological characteristics [7]. pH was measured using pH meter and the proximate composition was determined using the method of [5] **Statistical Analysis**:- Data were subjected to analysis of variance (ANOVA) and the difference among means was compared at 5% level of probability.

RESULTS

Total bacterial and fungal population and their characterization The effect of fermentation on the total viable count was variable (Table 1) and the total viable count of bacteria decreased with increase in fermentation hours as compared to the unfermented water yam samples, whereas the fungal population increased with increase in the fermentation hours (Table 1). The bacterial population of the raw (unfermented) sample was found to be 3.5×10^4 cfu/g, which decreased after 24 hours of fermentation (2.7×10^4 cfu/g) , which was observed to be lower than the raw sample. The bacterial count consequently decreased as the hour of fermentation increased. The lowest count (1.2×10^4 cfu/g) was found to be at 120 h of fermentation of water yam.

Two bacteria isolated from the raw samples were found to be *Bacillus subtilis* and *Staphylococcus aureus* whereas as the bacteria isolated from the fermented sample after 24 hours were *Pseudomonas aureginosa* and *Bacillus licheniforms*. *Staphylococcus aureus, Aerococcus viridians, Leuconostoc mesenteriodes and Bacillus subtilis* were isolated after 48, 72, 96 and 120 hours of fermentation of water yam respectively. The fungi population of the raw sample was found to be 7.0×10^4 spore/g of water yam which progressively increased as the hours of fermentation increased. The fungal population of fermented sample ranged between 8.0-10.0 $\times 10^4$ spore/g, the highest being after 120 h of fermentation (10×10^4 spore/g).

Table 1: Total microbial population (cfu/g) of water yam during 120 hours of fermentation. The bacterial population decreased as the fermentation increased while as the fungal population increased as fermentation increased.

Fermentation period (h)	Total viable count	
	Bacteria	Fungus
0	3.5 × 10 4	7.0×10^4
24	2.7 × 10 4	7.0×10^4
48	2.2 × 10 4	8.0×10^4
72	2.0 × 10 4	8.5×10^4
96	1.6 × 10 4	9.0×10^4
120	1.2 × 10 4	10.0×10^4

Table 2: The proximate composition (%) of raw (unfermented) and fermented sample of water yam. The moisture, lipid, ash and fibre content decreased as the fermentation increased while protein and carbohydrate increased with the increase in fermentation.

fermentation period (h)	Moisture	Lipid	Ash	Fibre	Protein	CHO(NFE)	pH
0	$3.66^d \pm0.01$	$6.31^e\pm0.01$	$4.91^e\pm0.04$	$9.31^e\pm0.01$	$5.76^a \pm0.01$	$70.03^a\pm0.02$	$7.14^a\pm0.01$
24	$3.62^d \pm0.06$	$5.90^d\pm0.02$	$3.90^d\pm0.01$	$9.75^f\pm0.01$	$6.02^b \pm0.02$	$70.74^b\pm0.02$	$6.34^b\pm0.01$
48	$2.61^c \pm.0.01$	$4.56^c\pm0.02$	$3.63^c\pm0.01$	$4.48^d\pm0.02$	$6.27^c \pm0.01$	$78.41^c\pm0.01$	$5.98^d\pm0.02$
72	$2.56^c\pm0.02$	$3.83^b\pm0.01$	$3.50^b\pm0.01$	$4.35^c\pm0.03$	$6.63^d \pm0.01$	$79.06^d\pm0.01$	$5.34^c\pm0.01$
96	$2.48^b\pm0.02$	$3.46^a\pm0.08$	$3.12^a\pm0.01$	$3.97^b\pm0.02$	$9.31^c \pm0.01$	$78.51^e\pm0.01$	$4.98^d\pm0.04$
120	$2.22^a\pm0.02$	$3.41^a\pm0.02$	$3.11^a\pm0.01$	$3.44^a\pm0.02$	$10.34^e \pm0.06$	$77.48^f\pm0.01$	$4.00^d\pm0.01$

Values are means of three replicates

Six species of fungi were isolated and identified from both fermented and raw (unfermented) samples. Three isolated from raw sample were *Aspergillus niger*, *Rhizopus stolomformis* and *Saccharomyces cerevisiae*, the three fungi isolated after 24 hours of fermentation were *Penicillium italicum*, *Fusarium solani*, and *Candida albicans*. One fungi was isolated each at 48,72,96 and 120 hours, they were *Aspergillus fumigates* (48 h), *Aspergillus flavus* (72h), *Fusarium oxysporium* (96 h) and *Penicillium italicum* (120 h).

Proximate composition of fermented and raw (unfermented) sample of water yam

The moisture content, lipid, ash, fiber, protein, CHO and pH varied (Table 2). The moisture content significantly ($P \leq 0.05$) decreased with the increase in the hour of fermentation as compared to unfermented sample. The lowest moisture content (2.22) was observed after 120 h of fermentation of water yam as compared to the moisture content of raw water yam (3.66). The 120 h of fermentation of water yam shows a decrease of 32.24% as compared to control (unfermented water yam). Similarly, the lipid content of water yam shows significant decrease as the hour of fermentation increased compared to unfermented water yam.

The highest decrease in lipid content was observed at 120 h of fermentation of water yam which was found to be 3.11 as compared to control (6.31). It shows a decrease of 102.9% as compared to unfermented water yam. The ash content of the raw sample was 4.91. The ash contents sample of the fermented for 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs were 3.90, 3.12 and 3.11 respectively. It can be observed that there was a significant difference between the raw sample and the sample fermented for 24 hrs, 48 and72hours but there was no significant different between the sample fermented for 96hrs and 120hrs. It was also observed that the ash content values decreases as fermentation progresses. The crude fibre value for the raw sample was 9.31.

A little increase was noted on the sample fermented for 24hrs (9.75), decrease in fibre value was however observed between 48 and 120 hrs with sample fermented for 120 hrs having the lowest value of 3.44 and there was a significant different between the values. The protein value of the raw samples was 5.76. The protein content of the fermented sample ranged between 6.02 (24 h) to 10.34 (120 h). From the study it can be concluded that the protein content increased with increase in days of fermentation.

The CHO content values of the raw sample was 70.3, while in the sample fermented, it ranged between 70.74 and 77.48. The pH value decreased as fermentation progressed and the lowest pH (4.0) was observed at 120 h of fermentation of water yam as compared to control (7.14).

DISCUSSION

Roots and tubers of water yam are usually consumed after subjected to various processing methods which may range from cooking to roasting and frying. All these processing methods result in an increase in keeping quality, palatability, digestibility, nutrient content and safety. During processing some nutritive values may reduce and some increase, while in some cases anti nutrient substances may reduce depending upon the processing methods [8] . However, from this study it is observed that the raw unfermented sample had the highest microbial population, compared to the sample fermented for 120 hours (5 days) which had the lowest microbial population. The decrease in microbial population may be due to increase in acidic values (reduced pH values) of the sample as fermentation progressed. Some microorganism may be inhibited under low pH condition.

The most predominant bacteria in the sample was *Bacillus* species isolated from both raw and fermented samples. This may be due to the fact that *Bacillus* species can thrive in various kinds and most numbers of foods, thus showing the ability that the spores can withstand different unfavorable environmental conditions. The pH of the sample decreased with increase in the days of fermentation, the microbial load also decreases as fermentation progresses. This shows that there was a relationship between microbial load, pH and time of fermentation. The decrease in pH values as fermentation progressed may be as a result of the activities of the microorganisms that are fermenting the samples which resulted in the breaking down of complex organic compound of the substrate where they produce acid and ethanol. Ethanol or acid is known to inhibit microbial growth in the fermenting substrate which are known to cease growth at certain level of acid or ethanol. The inhibitory effect of ethanol is due to denaturation of protein and also due to lipid solvent [9]. Since microbial cell wall is largely composed of lipids, ethanol causes dissolution of lipids in the microbial cell wall. Many proteinaceous foods of roots and tubers have been fermented by *Bacillus* species and several other fermented products rely on the participation of the various *Bacillus* species [10]. *Bacillus species* could be proteolytic, lipolitic and amylolitic depending on the type involved in the fermentation.

The moisture content in the fermented sample activated the proteolytic enzymes of microbes such as *Leuconostoc mesenteriodes*, likely to be trapped from air, *Aerococcus* spp, *pseudomonas* species and *Bacillus* species which are natural microflora of the water yam which in turn triggers the amylolitic enzymes and the hydrolytic enzymes which degrade starch to sugar and then ferment the sample [11]. The isolation of *Staphylococcus aureus* from the sample may be as a result of human contamination, because

Staphylococcus species are found as normal flora on the human skin.

In this study we observed that the fungal population increased with increase in the hour fermentation. The increase in fungal population may be due to decrease in pH as fermentation progressed, as acid pH is favorable for the growth of fungi which are mostly acid tolerant. Isolation of *Aspergillus* and *Penicillium* species in this study are observed to be the most predominant fungi showing that these fungi may be the normal flora of the water yam and thus they play a major role in fermentation, since the fungi are capable of producing amylolitic enzymes which break down starch to produce acid and ethanol, hence according to [12], *Aspergillus niger* produces citric acid during fermentation of starch.

The result obtained from the proximate analysis of the raw and fermented water yam sample indicated that there was a significant (P <0.05) change in the moisture, protein, ash, fat, crude fibre and CHO. The reduction in the moisture content as fermentation progresses may be attributed to the fact that decrease in the moisture content of the fermented substrate increases the chemical nutrient [13]. The results obtained showed that the decrease in lipid content was with increase in the days of fermentation, the samples fermented for 120 hours showed the lowest lipid content (3.41%). This may be due to the breakdown of fatty acid and glycerol by lipolitic organisms present in the sample during fermentation. The break down resulted in the increase of Aroma, taste, odour and texture of fermented sample [14], [15] Reduction in the lipid content increased the shelf life of food sample.

Our results in this study showed that the ash content of the water yam decreased with increase in fermentation of the water yam. The decrease in ash content may be attributed to leaching of the soluble inorganic salt during the processing of fermentation. [16]. It can be observed that the crude fiber content of the fermented sample decreased with increase in the hour of fermentation of water yam in this study. This may be due to the fact that during fermentation carbohydrates are broken down by fermenting microorganisms which are used as carbon source and converted to microbial biomass , thereby reducing the fiber content of such food [17] .

Increase in protein content observed as fermentation progressess can be attributed to great enzymatic activities of the microorganism involved in the fermentation. [18] reported that *Aspergillus niger* contain a number of enzymes that enables it to carried out many metabolitic activities. These enzymes include the proteases that can result in increase in protein content. There was an increase initially in the carbohydrate content , but later decrease in value was observed, this decrease was attributed to utilization of carbohydrate as energy source by fermenting microorganism, the carbohydrate are usually hydrolyzed to glucose and then use as source of carbon and energy for microbial growth. [19], [20] also reported decrease in carbohydrate content during fermentation of starchy substances

CONCLUSION

The results obtained in the microbial analysis during the natural fermentation of water yam revealed that different types of microorganisms where involved in the natural fermentation and that fermentation of the water yam sample had greatly helped in enriching the nutrient composition.

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